Kascak, Richard 2004

Dr. Richard Kascak Oral History 2004

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Dr. Richard Kascak Interview

Office of NIH History
Oral History Program

Interviewer: Maya Ponte

Interviewee: Dr. Richard Kascak

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Richard: [Beginning of transcript]. I had a fellowship at Cornell. I worked with an interesting group of people. They were working on Arboviruses. We were doing mostly Bunyamwera super group viruses looking at all of these little niches have their very interesting stories. In mosquitoes those viruses cause a persistent lifelong infection and when mosquitoes bite you they take a blood meal they transmit virus. And a lot of the viruses -- I mean, West Nile is a perfect example. Now, West Nile is not a bunionware super group, but it is a toga virus, so it is in that same family of viruses that replicate and transmit that way. When I was at Cornell we were studying those viruses, basically the differences between persistent infections in arthropods versus lethal infections in mammalian cells. I mean, why can a virus in one system be asymptomatic and in another system cause disease? Those were the kinds of things we were studying.

Interviewer: Are there things that you learned during that time that you feel have carried over and been useful in terms of thinking about or conceptualizing TSEs and what might be going on there?

Richard: Well, all infectious diseases have some common threads and I think once you get into the field of studying infectious diseases you can see similarities and sort of lessons that can be learned from all of these. I think SARS is an excellent example of how these agents can just sort of appear and doom and gloom kind of scenarios can be written, and you don't really know what's going to happen. Luckily for us in the world maybe, the SARS epidemic really turned out to be not as much of a problem as many people envisioned that it could be. But maybe we were lucky that the ability for that agent to be transmitted was controlled. If it hadn't been controlled in another way, perhaps things could have been different. I think there's a common thread that runs through all of these kinds of diseases -- again, whether it's SARS, West Nile, prion disease, there's lessons to be learned from all of them. Who knows, the final scenario of prion diseases hasn't been written yet. This is a slow disease and the story is still slowly developing.

Interviewer: How did you first get involved in the field?

Richard: I actually came to this institute as a postdoc on a project studying amyotrophic lateral sclerosis and post-polio syndrome. In those times, and that was 1976, it wasn't known what the cause of amyotrophic lateral sclerosis was. It was thought that it could be a viral infection. Certainly similarities between ALS post-polio syndrome and polio made people think that maybe amyotrophic lateral sclerosis could be caused by an infectious agent. So we had an NIH-funded project to study whether or not we could identify any virus in tissue from patients with amyotrophic lateral sclerosis. And we found a few viruses but nothing turned out to be the cause of the disease. Now there's a lot more known about amyotrophic lateral sclerosis, but in those days, a viral etiology was thought to be one of the likely scenarios.

Interviewer: And what kind of things were you trying to do to try to find a virus in that case?

Richard: We did a lot of things. Mostly we did autopsies. In those days autopsies were much easier to do, especially before the advent of HIV. So a Dr. Donafeld [spelled phonetically], who was a neural pathologist at Saint Vincent's Hospital in Manhattan, and I tried to do autopsies within one to two hours of death. We sort of went on a moment's notice and it was mostly in the New York metropolitan area, sometimes in New Jersey, sometimes in various parts of New York. Because he was a neuropathologist, he did the autopsy and I collected tissues and we cultured cells. We cultured brain cells, serum, CSF and did a whole variety of studies looking for some evidence of a virus etiology.

Interviewer: Did you do any animal inoculations?

Richard: Yes, we did animal inoculations.

Interviewer: Okay. So you tried the whole gamut?

Richard: We did -- We did a lot of animal inoculations here. Again, Dr. Carp was also part of that project so we had the animal colonies, and were injecting mice and hamsters and seeing. It was a real fishing expedition, but nobody knew what would happen. But that was the reason why I came here. Because I minored in immunology at Cornell, I had strong background in immunology. In studying these diseases, it became very clear that immunoassays were the most sensitive way to look for a virus -- whatever you're going to look for, doing it immunologically is much more sensitive than taking a slide and looking under an electron microscope and trying to find a needle in a haystack. We developed a lot of immunoassays. We were doing all kinds of ELISAs and neutralization tests and things like that when we were doing the ALS studies. I became interested in developing monoclonal antibodies for some of the agents that we were looking for. Once we started doing that we became involved in a variety of things. Because there was much work at NIH on Alzheimer's disease we became interested in making some monoclonal antibodies to the paired helical filaments. That was one of the first antigens that I used to develop antibodies.

Interviewer: And what were the challenges in doing that? If you can tell me a little bit about within the process of raising monoclonal antibodies, what are some of the steps that are difficult or challenging?

Richard: Well, one of the main problems with making monoclonal antibodies to many of these proteins is that they are abnormally processed normal proteins in animals and in humans. Therefore, animals can be tolerant to these proteins. They see them as self. They don't make an antibody. That's one of the problems with PrP and many different proteins. In the early days we had to think of ingenious ways of trying to make antibodies to some of these proteins.

Interviewer: And what would you do? What sort of techniques did you develop get around that difficulty?

Richard: One thing you can try to do is to present the antigen in a way in which it will not be seen as self. For the paired helical filaments, we could inoculate paired helical filaments and we were able to get good immune responses. In those days most of the antibodies we generated turned out to be to ubiquitin. I don't know if you know the whole ubiquitin story. In those days not very much was known about ubiquitin, but now ubiquitin has become the major element in protein processing. Many proteins designated for degradation are ubiquitinated. Some of the antibodies that we developed initially to paired helical filaments have now become very useful in studying the ubiquitin pathway processing of a whole range of proteins.

Interviewer: At the time you didn't know that they were antibodies to ubiquitin, you just knew that they were reacting to –

Richard: No, that -- paired helical filaments, right.

Interviewer: That's really interesting. So then PrP, how did you then get involved in that story?

Richard: Well, because Dr. Carp was head of this department. Alan Dickinson and Richard Kimberlin are very good friends of Richard Carp. So I met all of those people and it sort of worked out that I studied the problem.

Interviewer: Would they come visit?

Richard: They would come and gave seminars after which we talked.

Interviewer: So you started --

Richard: If you want to get a true introduction into the TSE field -- it was always called TSEs, it was never called prion diseases -- that those were the perfect people to have that introduction. Alan Dickinson and Richard Kimberlin would come here; we made a few trips to Edinburgh. And in the mid '80s it became evident that people needed a more efficient way to diagnosis these diseases. We started out -- Paul Bendheim and Dave Bolten both came from Prusiner's lab. Paul had started making some polyclonal antibodies to PrP. We also made a series of polyclonal antibodies in rabbits to PrP that were very useful. Some of those antibodies are still used today. I mean, we had a real stockpile of these and stored them in freezer. Some people still love to use those antibodies rather than some of the monoclonals that are commercially available.

Interviewer: Can you talk about a little bit about the advantages and the disadvantages of monoclonal versus polyclonals?

Richard: The advantage of a polyclonal is that most polyclonals are made in rabbits. Rabbits, even though they're a mammalian species, view many of the antigens that we're dealing with differently than do mice or rats. What happens in rabbits because their PrP gene -- the immune response to PrP is mostly controlled by tolerance, what animals view as self and non-self. The sequence of the PrP protein in rabbits is sufficiently different from that in mice, rats and hamsters so that many of the sites on those proteins are seen as foreign by the rabbits so you'll get a wider repertoire of responses. In many rabbit sera you can do epitope mapping. At least the linear epitopes of many of these antibodies are relatively easy to map. The discontinuous epitopes are much more difficult but linear epitopes are relatively easy to map.

Interviewer: And what's the difference between linear and discontinuous?

Richard: Discontinuous is a conformational epitope.

Interviewer: Discontinuous is conformational, they're near each other because of the way it folds and continuous -- linear is based on primary sequence.

Richard: Right, it's based on primary sequence.

Interviewer: Okay.

Richard: So primary sequence epitopes, linear epitopes are relatively easy to map. We use a procedure call pep-scanning and you look for reactivity and you change them one amino acid at a time so you can monitor the epitope size and which is recognized by the various antibodies. Many of the rabbits responded to 8, 10, 15 different epitopes when we immunized them.

Interviewer: You mean that you're developing these polyclonals so what you get is a mixture of antibodies that are going to recognize different epitopes and certain species quite well because the PrP is different?

Richard: Right. The problem with rabbit antibodies is that they can be very useful, but you're limited in the amount that you can make because you bleed the rabbit; we usually do it through ear veins and we can collect maybe 20-30ml of blood at a time. But the response in the rabbit changes over time. So even though you keep immunizing the rabbit and keep collecting antibodies, the antibody repertoire in the first bleed may be very different than the antibody repertoire in the fourth bleed. Also, you can't control what's called the avidity, Kd. Many antibodies today are measured by their Kds, how well they bind to the antigens of interest. Those polyclonal antibodies have a mixtures of Kds; not only of different epitopes, but different antibodies have different Kds. So it's hard to control. You're limited by this mixture and by availability.

Interviewer: Right, and variability like you're saying of the mixture may be a different.

Richard: In today's world where antibodies really serve as a major diagnostic tool, you need a large amount of antibody to be able to do this. Monoclonals are the ideal solution. You can select for antibodies that have high binding constants -- you measure them in the negative range, so low Kds. Like 3F4, 3F4 has a Kd of 10-11. That's why that antibody has been so widely used. It has a very high affinity for PrP. It doesn't see anything else and it can find that one PrP molecule in a sea of thousands of other proteins, so it's been very useful because of the Kd. The other advantage that monoclonal antibodies have is once you have the clone you can make antibody forever. 3F4 was made in 1990 when essentially the first clones were generated and the ones that are now being made in 2004 are exactly the same. There is no difference in the antibody.

Interviewer: So it's much more of standardized reagent in a sense?

Richard: Yes.

Interviewer: And also you get very low background noise.

Richard: Right. We try to select for antibodies that have a very low Kd, that have a very high binding constant, which makes them very useful in a whole range of different amino acids.

Interviewer: Are there any limitations to monoclonals or any reasons why you would sometimes choose a polyclonal over a monoclonal?

Richard: One of the reasons would be the ability to generate the antibody that you want. I will give you an example with tests that we are performing in collaboration with Signet Laboratories.

Richard: They recently came out with a diagnostic kit for Alzheimer's disease. The kit uses a combination of monoclonal antibodies -- monoclonal antibodies that we supply…but also they use detection antibodies that are rabbit polyclonals. Those rabbit polyclonals can distinguish between what's considered 1 to 40 and 1 to 42. The 1 to 42 is the processed form that forms the amyloid plaques. Another collaborator at NIH has generated the polyclonal antibodies that can distinguish between 1 to 40 and 1 to 42. So in the capture ELISA assay that Signet has developed, they use our monoclonals to capture and his polyclonals to detect. So in instances like that where you need -- where you are looking for a specific kind of reactivity in certain situations where the monoclonal doesn't exist. If at some point we are able to develop monoclonals that have the same properties as the polyclonals, then our monoclonals would be used instead of the polyclonals

Interviewer: So then back to TSEs. You started working a little bit with Hino when he came and you're trying to identify some protein component that seems important. Tell me the story of what happened next.

Richard: What happened was that Hino was also making some polyclonal antibodies. We were making some polyclonal antibodies and Hino said, "Why don't we try to make a monoclonal?" Again, we had the experience in making monoclonals through other proteins, so we knew how to make them. So we decided, "Well, let's make some monoclonals to PrP."

Interviewer: Did you think it was going to be difficult? I mean were there any concerns?

Richard: At that time we didn't understand all the issues of tolerance and what really controlled the immune response. The immunogen that was used to make 3F4 was actually hamster PrP. So we immunized mice with -- and we used standard, BALB/c mice because they were used in those days to make monoclonals because most of the myeloma lines used as the fusion partner are derived from BALB/c mice, so we immunized BALB/c mice with hamster PrP. As it turns out, there's a sufficient sequence difference between mouse and hamster PrP, that even though the mice would be tolerant to any mouse epitope, they were not tolerant to hamster-specific epitopes. We made a variety of antibodies. One of those was 3F4, which recognizes hamster -- actually 3F4 recognizes three species: hamsters, felines, and humans with comparable Kds. It doesn't recognize in any other species. It only recognizes those species and we know why now because all these genes have been sequenced. The epitope for 3F4 is a linear epitope. We know what the epitope is and you know, sort of through serendipity that the epitope is unique to those three species.

Interviewer: At the time you couldn't have known that?

Richard: No.

Interviewer: There was no way to know that because the sequences weren't known.

Richard: Yes

Interviewer: I mean, this was all very new at the time. So basically it was kind of luck or serendipity in a sense that the hamster, for whatever reason, that particular clone.

Richard: Yes, and we have other antibodies that came out of those studies that are not as good as 3F4.

Interviewer: So what was the process of elimination of like? What did you do then to select 3F4 from the different clones that you established?

Richard: When you do a fusion in the beginning, any clone that specifically reacts with your immunogens you save. So 3F4 was actually one of the first clones that grew out of that fusion and it looked to be a very good antibody from the very beginning. So then you went through and characterize it.

Interviewer: What you had of the pure -- partially purified hamster?

Richard: Yes. There's a whole process, you have to clone these, so I've cloned them, froze them down, stabilized the clones, but once all of that is done and you have a sufficient amount of antibody to then start characterizing. The first question was how good is this antibody? What kind of immunoassays can use the antibody to perform and what different species of PrP does the antibody react? It turned out to react very well to human PrP. But as many people that have used it for human PrP, probably an equal number of people have used it to study hamster PrP.

Interviewer: What were the offshoots that developed? So once 3F4? Can you talk a little bit about sort of your philosophy? One thing that I've learned a lot from spending time in the field is that your group was very good about sharing. Can you talk a little bit about your philosophy behind handling of the antibody and making decisions about how available to make it?

Richard: The world has changed because of the biotechnology industry. We're continuing to make antibodies; as I said before, I run a monoclonal antibody lab now. We market many of these antibodies through Signet Laboratories. So we are very willing now to give people antibody. Once we've developed an antibody that's useful, if people doing research want to use those antibodies, we're more than willing to supply them with as much antibody as they need but we will not give out the hybridomas. Initially, with 3F4, we did do that and in fact I have a stack of papers this big of people, Stan Prusiner being one of them, that signed an agreement about restrictions of how they could use 3F4. Stan hasn't abided by those restrictions, unlike most of the other people that have used them. But I gave the antibody to Collinge also, almost all of the work that Collinge and Hill have published on the different forms of PrP, especially their being able to distinguish variant PrP from other forms of sporadic CJD/PrP was all done using 3F4. But that's okay

Interviewer: Well what kind of restrictions supposedly did they sign?

Richard: I could give you a copy of the letter of the agreement that they signed, but basically the letter says that you are free to use this antibody for research purposes. If you develop a test or market this for commercial purposes where revenue is generated, we deserve to be compensated for your use of our antibody. Now, having that signed and having that enforced is another issue. Signet is working on some of these issues, because of the popularity of 3F4 and its use by many different people. Out of curiosity, last week someone from Signet asked me to do a Google search for 3F4. How many times is it referenced? It came up with 780 references in Google for monoclonal antibody 3F4. Some of those are my publications, but not all of them. Many people have used that antibody. And that's also helped to do collaborations because we've collaborated with a lot of people in using that antibody. And that's another good thing that has come out of the monoclonal antibody facility is that we make these reagents and it allows us to collaborate with many people. I gave a talk the other day about involvement of IBR [?] with the biotech industry, and companies want to do things quickly and the availability of resources like antibodies and expertise helps these companies get their product developed much more quickly. So we work now with a lot of different biotech companies to help develop their products. Most of that not only involves our expertise on prion diseases but also involves our antibodies.

Interviewer: Right. And like you're saying too, it helped form collaborations with many other groups in the field. Do you think that it's one of the things that has helped the lab to become involved in other types of studies?

Richard: Oh, most definitely.

Interviewer: And what are some of the collaborations that you would cite as the most fruitful based on sort of shared resources?

Richard: We've collaborated with a great many groups. We know -- Bob Roure [spelled phonetically] we have a contract with him. We've collaborated with him for years when he was in Gibb's [spelled phonetically] and Geidershek's [spelled phonetically] and is now in his own lab. Claudio Soto, we did work with Claudio. Claudio now has developed his PMCA assay. Much of the early work that Claudio did when he was at NYU involved our antibodies. We've done a lot of work with Robert Summerville [spelled phonetically and John Philippe Delise [spelled phonetically]. John Philippe stayed in my house for a month. He came here when he didn't know what a TSE agent was.

Interviewer: Wow. [laughs]

Richard: And so he stayed with me. Regina showed him how to purify PrP and his first introduction to this field was here. Now he's a major player, he and Bio-Rad, in PrP diagnostics.

Interviewer: Many connections there. And what have been the major offshoots of having a successful monoclonal ? What has that led to in terms of other technologies within the field?

Richard: Well again, as I said, monoclonals are very specific and sensitive tools for following these proteins but technologies develop all the time. New assays and technologies become available, again, collaborations now with Q-RNA is developing on two lines. One is they're developing what they call Amplibodies, which are RNA type molecules that specifically bind to PrP. But that's only one part of the story. The other part of the story is to use those reagents as a diagnostic tool for sensitivity because using the Q beta replicase you can generate a tremendous signal by having that RNA molecule tagged to your antibody. So that's what we've been working on with Abraham Grossman to use two monoclonals, one as caption, one as a detection, of the second antibody tagged with the -- what he calls RQ11-12, use that and then use the Q beta replicase to amplify that signal. You can generate a tremendous signal with that kind of an approach.

Interviewer: What exactly is Q beta replicase?

Richard: Q beta replicase is a replicase that replicates Q beta RNA. Q beta is an RNA phage. Abraham Grossman has found that certain regions of that RNA specifically bind to PrP, and you can use that binding capacity. Now, there's two sides to that story, again, as I said. One of that is you can use what he calls RQ11-12 to bind to PrP and then use that as part of the diagnostic assay. But the other side of that coin is just using the ability of Q beta replicase to replicate RQ11-12. And you can put a tag on, I mean you can if you want to do it radioactively, if you want to use a non-radioactive probe to be incorporated into that RNA you can then detect that signal. And its ability to generate such a large amount of that RNA over very short periods of time makes it a tremendous diagnostic tool. The problem in this field now, as from the diagnostic point of view, is sensitivity. That's what everyone is working on. The level of infectivity in blood, and blood is the major issue, is low. I mean, even for 263K which has -- in brain has a level of 10^9th infectious units per gram. There's only about ten infectious units per mL of blood. So how do you detect those low levels of material? And 263K may even be at the high end of that spectrum. You may only be talking many of these conditions like BSE or varying CJD, only one or two or a handful of infectious agents per mL of blood. That's what you need to be able to detect.

Interviewer: Why is it blood that's the focus?

Richard: Because it's the most easily obtainable specimen. You can obtain blood from yone. Blood is taken from people all the time for all kinds of reasons. So it's the most available diagnostic specimen. In addition, blood is used in the pharmaceutical industry for many products. The studies that we're doing us all kinds of blood fractionations; factor 8, factor 10. I mean all these different factors in blood are used for a variety of purposes. They have to guarantee that that material does not contain prions. So they're very interested in monitoring their material, and again, liability is driving a lot of this. If a sample that they use to treat a child contains one infectious unit of these agents, the child will die. It may take five years, but that child will die. So that's a liability that industry. Let's consider gelatin. How many pounds of gelatin does everyone consume a year from all kinds of vitamin capsules whatever? Gelatin is derived from bovines, from cattle. What's the possibility that the gelatin, if it came from a BSE infected animal, contains prion infectivity? So this is a tremendous interest to the companies that are making these pharmaceutical products. So they want to make sure from a liability point of view that as best as it can controlled that there is no infectivity associated with any of their products. So that's what's driving a lot of these studies these days, is to ensure, especially with the variant CJD because of the different properties of that agent compared to sporadic CJD.

Interviewer: With the pharmaceutical companies there seems to be a very high level of concern. Sort of what you're talking about, partly just to avoid liability for if there is any spread, but do you see that same level of concern in the agriculture industry?

Richard: Well it depends. There's a lot of interest in CWD. There's a lot of interest in scrapie. At the moment there is no concrete evidence that those diseases are transmissible to humans, especially scrapie, because BSE originated in Great Britain. People had been living in very close proximity to scrapie-infected sheep for hundreds of years and there was never any evidence -- now people may say that it could be there at a very, very low level although it would be very difficult to distinguish, but as best as anyone knows it has been -- there has been no evidence that suggests that scrapie can be transmitted to humans. But now with the advent of BSE, a disease like chronic wasting disease is certainly of interest. Chronic wasting disease was not very important because it was largely in the middle of the woods. Elk or the mule deer would die but who cares? But now that the human population is infringing upon the areas, and now with the white tail deer also being shown to be susceptible to CWD, which –

Interviewer: Bringing -- so then the whole country sort of becomes implicated…

Richard: Right, and certainly the white-tailed deer tend to be more closely associated with populated areas than are either the mule deer or the elk, but even those now, because people are infringing more and more on their territory there is a concern that there will be more and more contact of humans with these animals. And what is the possibility that a BSE-like situation could occur with CWD? Nobody knows the answer.

Richard: I don't know if she's made any judgments. I think she's letting science develop this and she's certainly working with a lot of people now in trying to answer that question, what is the possibility of CWD being transmitted to humans? I'm not sure if BSE hadn't occurred whether anyone would be asking those questions, but now with the transmission of BSE not only to humans, but a variety of other species, there's also much interest in trying to address that same with CWD.

Interviewer: I wonder if you could tell me a little bit more about the breadth study and its goals and where you get the different tissues from?

Richard: That grant was essentially funded to develop a blood-based diagnostic assay for TSEs. They would like to focus on human disease but because of various restrictions we're doing it with other -- with some of the mouse models and also with sheep. We're working with Marie Volgen [spelled phonetically] at the University of Idaho. Do you know Marie?

Interviewer: Yep, I know Marie.

Richard: Marie has been supplying us with tissue samples and blood samples from infected sheep and we've been working on blood-based diagnostic assays.

Interviewer: And are you able to get enough tissue -- I mean, are you able to get enough samples from that source for Marie Volgen's scrapie?

Richard: Yes. She has both naturally infected and experimentally infected animals so there's sources of material from either natural animals or from infected animals. So she has been able to supply us and we've been looking on two fronts. Chen Wa Wang is trying to work out an assay where we could detect the PrPSc in plasma. He's working more on the plasma side. I'm working more on blood. We've been using the filters which permit separation of blood cell components. So we've been looking at blood cell components using the filters to remove the PrPSc associated with the cell components, and that's worked out very well.

Interviewer: So you're working on both detection and removal technologies?

Richard: Yes.

Richard: We have an idea of what they call different chemistries and structures of filters that had been used for a variety of infectious agents. We wanted to see whether they could be used to remove prions. We did a series of experiments with them looking at removal. The removal studies were done as spiking studies. We took hamster brain from 263K-infected animals and spiked it into their human blood samples, blood cell samples. We made brain homogenates. We diluted those down to a final of 1% in their blood, the human blood that they were using, and we tried a variety of filters to see if any filters would specifically remove the PrPSc. And it turned out that it did. And we also found out that the filters were relatively specific for PrPSc, They don't work very well in removing PrPc, but they removed PrPSc,

Interviewer: What's your thinking on why that would be?

Richard: It has something to do with the chemistry of the filter. We don't really know. Paul knows much more about the chemistry than I do but it has something to do -- we feel it's a combination of chemistry and structure. There's a structure to these filters. They make them in a whole variety of different ways -- I mean you have to talk to their chemist, but it's a structure in chemistry of that filter that defines it's properties, and it was found that two of their many, many, many different kinds of filters that they had tended to bind PrPSc.

Interviewer: So you really -- in a sense you just went at it like you had all these different filters that they had developed like a screening assay almost?

Richard: Right, and Gina just did this. We screened a whole bunch of filters in the beginning and we found that a couple of different filters worked relatively well.

Pat: This is pure blood with cells?

Richard: This is spiked blood cell components. We removed the plasma and kept everything else. We kept the buffy coat. We kept all the cells. The only thing we removed was the plasma.

Richard: …the red cells go through filter and get the PrP.

Richard: Right, we started out with everything except the plasma.

We found certain filters worked very well. At that point we said, "We basically if the stuff is being removed by the filter, it has to be on the filter. So let's take it off the filter and use it as a diagnostic assay."

Interviewer: Nice.

Richard: Paul said, "Oh that's a good idea." So now if you go to Paul's website all they talk about is the detection. So that's what we're doing now. We're trying to refine the ability to use those filters as a means to detect PrP. It's a tremendous concentration step because we can filter a liter of blood through this filter, remove all of the PrP that's bound to it and bring it up at 100 microliters and test it.

Pat: That's spiked PrP.

Richard: Well, we've also done it with endogenous.

Interviewer: Oh now you've done it with endogenous too.

Richard: We did do it with endogenous --

Interviewer: -- with like Marie Volgen's sheep blood.

Richard: Right, we've done it with it hamster 263K infected endogenous blood and sheep blood and under both of those instances -- actually Regina did a blind study. Marie sent us seven coded samples and we only got six out of the seven right. The one animal that we got wrong was an animal that was very, very ill and was almost dead when they removed the blood. So we don't know if that affected the ability to do the assay.

Interviewer: So are there plans to do bigger studies?

Richard: Yes.

Interviewer: Bigger validation studies now.

Richard: Right. We're in the process of arranging that.

Interviewer: How many samples do you think you need tto be convincing for like regulatory bodies?

Pat: Right that's the company's problem.

Interviewer: That's great then you can punt that. That's wonderful.

Interviewer: Well, now I guess this is a good question to ask with all of you here, this can be a free-for-all question, which is how has your sense of a model for the agent evolved over time and your thoughts on what adaptation is.. We talked about this a little bit, but if you -- so if you could kind of tell me first what you think –

Richard: Well, I think there's probably a uniform belief in the group that it's almost impossible for PrP alone to be responsible for all of the variation in strains that we see. The different biological properties would be ver difficult to explain. One of the reasons we argue against Stan's conformation-only hypothesis is you can do a lot to this protein. You can treat it with all kinds of chemicals. You can remove all the carbohydrates. You can remove the disulfide bonds. You can chop off the N-terminal. You can chop off the C-terminal and stuff still retains its biological properties. If this was strictly conformational you have to believe that doing all of that has to affect the conformation of that protein and yet its biological properties are unchanged. So it' s very difficult. Uri Safar and Stan now say that only eight are sequences in the protein are important, so you haven't really affected those. But I think a lot of people still find that very difficult to believe. And that's where there's still…

Pat: What people? What people find it difficult to believe?

Richard: Well, I mean certainly the Edinburgh group. Certainly people like --

Pat: It's those that work in TSEs.

Richard: Yes.

Interviewer: Why does it remain -- why does it even remain the same strain proprieties and stuff? You would think at least it would change strains or something.

Pat: Why is 90% of it sensitive? This is one of Rick's old lines. When you do your isolation procedure basically you only recover 10%. So why are the other 90% sensitive? And what have you done when you isolate it that has inactivated the whole 90% and you're only left with 10%? Is it that the 90% moves around freely and you're left with the most resistant portion of infectivity that you would ever see? Are you dealing with only a minute portion of the whole structure of the agent? These questions can't be answered until you decipher the 10%.

Richard: But I think part of what Claudio Soto has done also may shed some light on this, because he can generate in vitro molecules that biochemically mimic PrPSc and yet they're not infectious. So why not? By their antibody reactivity, migrations on gels, and pK sensitivity they look exactly like PrPSc, but they're not infectious. So why aren't they?

Interviewer: With the new language that Prusiner is using there's the sensitive and the resistant forms.

Richard: 139A is known as one of the most sensitive strains of the agent. This appears also some of the sheep strains. 139A is an interesting agent, because in mice it has one of the shortest incubation periods of any mouse strain and yet it's the most protease sensitive and doesn't produce amyloid. Yet, according to Stan's hypothesis, you would think that the agent that was most protease sensitive would have the longest incubation period. But it doesn't.

Interviewer: That's interesting. In hamsters with hyper-drowsy there it is the one that is more sensitive and has the longer incubation period, but here you're saying that –

Richard: It's the opposite.

Interviewer: In mice it's the opposite.

Richard: It really it is the opposite.

Interviewer: So there's something else going on.

Richard: Yes and that whole hyper-drowsy story too, because hyper is 263K and 263K is sort of the odd man out. I mean 263K has been very useful because of the high titers and high amount of PrPSc, but it's not.

Pat: It's not a typical agent.

Richard: No, it's not a typical agent. It doesn't have properties that are similar to most of the other agents that we deal with. It's an excellent tool, but it's not a great model.

Pat: Doesn't 263K -- if you re-inoculate it into mice and did blind passages over a couple of times you recover out a mouse strain?

Richard: Yeah, it's very difficult to --

Interviewer: So it's, but you said it's not typical, it's -- what's different between 263K and most strains, most mouse strains or --

Pat: Incubation period is minute, titer is gigantic, non-infective portion of PrP if you want to call it or SAF or the agent is gigantic so it's very -- pK resistance is gigantic.

Richard: And it also has a very low peripheral involvement. Titer in spleen is like three logs compared to 109th in brain. So the agent has a relatively restricted non-central nervous system implication.

Pat: If you compare some of the mouse strains that go to about 107th in brain, go a maximum 106th in spleen and then they -- at their peak, but it's basically really -- it's not the low level in the spleen, it's the low level in comparison to the amount that's in the brain.

Richard: Right, but still the level in the spleen in 263K hamster is logs lower than it is in most mouse strains.

Pat: No, only in comparison with brain.

Richard: So it's not a good model for what's going on in --

Interviewer: Right, I see what you're saying. It's really useful for like -- for what they did in terms of purifying this whole thing.

Richard: Yeah for the [inaudible] of PrP.

Interviewer: Right, and it's useful in studies because you get the short incubation period and you get this high infectivity coming out --

Richard: But that's changing now too with the transgenic animals because a lot of the transgenic animals are –

Interviewer: Oh I was going to ask you about transgenics.

Richard: They are overexpressing PrP can have relatively short incubation periods.

Pat: What's the question about transgenics that you have? I know my view on them.

Interviewer: I have a couple of questions. One is how useful of a model -- okay, there's two things because as we're talking about here with 263K there's the utility as a model that represents something in nature that you can say this -- what we've studied now with this model system is telling us something about how it operates in a natural host. Then there's utility for as a certain experimental tool perhaps, and so how do you view transgenics in light of those two different --

Richard: Well, I'll tell you my view with transgenics. We are very much involved in the immunoassay part of this, but immunoassays are not the answer unless you can relate them to infectivity.

Pat: Right, well I was going to go a different route than you are.

Richard: An immunoassay is only as valuable as you can relate it to infectivity. So if you can get x-signal from an immunoassay how does that relate to the amount of infectivity that's there, because that's what you have to guard against –

Pat: Watch your detection limit, its comparison to infectivity –

Richard: A company doesn't care how much PrPSc is there if that's not going to cause disease. They're concerned about how much infectivity is there. But immunoassays only detect PrPSc. So we have to have a mechanism of relating PrPSc in our immunoassays to infectivity, and transgenic animals are the best way of doing that because of time.

Pat: No, no, no, no. They're better than that. They're much better than that. That's why I put in because you're talking about diagnostics.

Richard: I'm talking about diagnostics.

Pat: Yeah, and you moved into infectivity. The best -- if you have the right constructs in the animals expressing at a relatively decent level -- of course that basically their normal PrP, they're the perfect animals to test whether or not any of the diseases when you have a new disease too. How fast does it mutate? How fast does it change? Will it ever infect the human population? You can't do these experiments in the population. That's why I -- yeah because this is different from immunity.

Richard: Right that -- right that's two sides of the transgenics. Yeah, obviously the issue that we were talking about of can CWD be passaged to humans? Can CWD be passaged to –

Pat: Well, make an inoculate [inaudible], I'll test you out.

Richard: Yeah. Right. So transgenics, although maybe they're not the perfect answer, I don't know if every result in a transgenic animal will mimic exactly what will happen in that species –

Pat: No, but they'll give you an indication.

Richard: -- but they will certainly give you an indication. So –

Pat: Stan was right about that.

Richard: -- if you want to find out whether CWD can be transmitted to humans -- I mean this is what Telling [spelled phonetically] is doing. Telling has got all these grants now trying to do these studies.

Pat: But I thought Telling didn't have -- he didn't have the right construct then. He didn't get any take.

Richard: No, I think now he does.

Pat: Because he put Chow's [spelled phonetically] construct in?

Richard: I don't know exactly what he has and what lines he has. I don't know like some of the lines that Rich made –

Pat: Yeah I know.

Richard: -- whether he has any of those now, but he certainly has enough transgenic lines where he's asking some of those questions.

Pat: And he has enough positives to be able to ask that, because previously he didn't have positives.

Richard: I don't know what his -- to be honest with you I don't know what his UDRL [?] white tailed deer transgenic situation is right now.

Pat: About a year or two -- it was a year or two ago he had nothing.

Richard: I honestly don't know the answer to that.

Interviewer: But the idea is that that have they that utility –

Richard: But that's basically the premise of using the transgenics, that you can do basically an experiment that you can't do without them or an experiment of an experiment.

Interviewer: Well how about issues of interpreting -- so okay, so then you have let's say the human transgene in there, but also granted it's still a mouse body with mouse organs and mouse tissues. So then how do you then -- if you get result whether it's positive or negative in the transgenic mouse.

Richard: Yeah, and I think that you're asking a very good question because some of the initial studies with BSE by Collinge was done into transgenics expressing human PrP and he found very long incubation periods and he was saying well, maybe this isn't so dangerous to humans after all. Well guess what? [laughs] So you have to take with a grain of salt all of these results.

Pat: -- well the other thing is what the isotype is, and whether or not you'd need two or one, whether or not you'd need -- it has to be recessive or not. So if you see methionine -- all of those.

Richard: So, I mean I think --

Pat: I mean, now you can set those up anyway you want -- once you get a take you can move those in and out.

Richard: Well yeah, and as these new reports about the methionine/valine you know where is this all going? But I think transgenics still have a role whether if you don't get transmission as Collins did in those initial studies. I think there were other reasons because they didn't quite understand the system, maybe the inoculum wasn't the right one, maybe the titer of infectivity in BSE brain is exceedingly low. I've heard estimates of only about 103rd infectious units per gram of tissue in a BSE-infected animal compared to 109th in a 263K and obviously titer plays a role in transmission. Well some of those –

Pat: That raises very interesting questions on pathogenicity of the -- it's not just the protein pathology, which again is a [inaudible]. It's not just protein pathology.

Richard: Yeah, because the amount of PrPSc in a BSE cattle brain is very easy to identify but still levels of infectivity are relatively low. So there's not the same -- if you compare that to a sporadic CJD case, the quantitative relationship between the amount of PrPSc and the amount of infectivity is very different. So what's controlling that? Why is the level of infectivity so low in the BSE? We don't know. But you can orally infect -- you can infect a hamster with one infectious unit orally. It may take 300 days for the animal to come down, but when the animal dies, it will have 109th infectious units per mL of brain homogenate. So I don't think that's the whole issue.

Pat: Well, the lesion profile is different. The lesion profile is hindbrain if I remember right, which would be -- is lethal.

Richard: That's another very interesting part of this story. I don't know if you've spoken to Maura Breifs [?], but the whole system that she's worked out is exquisite and tells you a lot about the biological variation of these agents.

Interviewer: What do you think is going on with strain competition, strain adaptation, when you move strain from on host to another?

Pat: Ah, that's easy. Do you want discussion of that? I put in two agents, one by the IP route and one by the IC route. The one I put in by the IC route doesn't particularly care for this animal to replicate in. The one I put in by the IP route likes this strain of animal. IP takes off and closes off IC. It doesn't destroy IC. It doesn't have to destroy it. It can allow recombination. It can shut it off by occupying sites early. It can resorb.

Richard: No, no I agree. I'm not sure I would use the term resorb.

Pat: Well combine.

Richard: You certainly get adaptation.

Pat: There is two-fold adaptation. One is if there is nucleic acid, that's my belief and background. Adaptation is two-fold. One, the RNA has to adapt to the protein that's present. That's number one. Number two, that RNA replicates up the ying-yang, but if it can't fit to the PrP you're not going to get it. You're not going to get the disease. So the adaptation -- the RNA can just go anywhere in its replication and in its base changes, but it has to fit the PrP. That's your long incubation period when you cross species, and that's why when you go a second time, it's a very short incubation period. So also the explanation for a mutation, because somewhere in that jungle of RNA will be one that's changed in a way the infectivity and lesion profiles change.

Interviewer: And then that gets selected out?

Pat: That gets selected out.

Interviewer: Okay. And do you pretty much agree? Is that also how you see it, Richard?

Pat: Oh he sees it much more scientifically than I do.

Richard: Yes, I'm not making any preconceived determinations about what is happening. I certainly believe that there is some associated informational molecule. It could be kryptonite. I have no idea what it is, but it's not PrP. It could be anything.

Interviewer: So you'll say it's RNA?

Richard: RNA. Yes.

Interviewer: Pretty definitely. Neither of you guys will put your finger on anything?

Interviewer: That's an important point, yeah.

Pat: It's a very important point. But if you're doing the work on it then you have to build frameworks under which you can test and a framework under which you can look at other people's work.

Richard: Yes I agree.

Interviewer: So that's where a model for how the agent works becomes important in terms of that framework, that evaluative framework.

Pat: It doesn't mean that your model is correct. I don't mean it that way. And if you ever believe that you're just so egotistical and narcissistic that you're not a scientist, but you need something to go against and to say that's where the no's are important. The yes's don't mean anything. They just reaffirm what you believe. It's the no's that are important.

Interviewer: Right so one should in essence seek to disprove he model is?

Pat: I always seek to disprove.

Interviewer: Well that might be a good ending. [laughs] Anything else to say?

Richard: Just one other thing on the strains. Did you talk with Richard Kimberlin?

Interviewer: I did.

Richard: Yeah, but did he talk to you about his very early studies in which he took agents, passed them into hamsters, mice and rats?

Interviewer: Yes, I'm familiar with those.

Richard: Those are very interesting studies because he found some agents that no matter what you did to them when you put them back into mice they were exactly the same as what you started with. But other agents were very different and he in fact created some agents that never existed before he did those passages. So those -- again, before you knew anything about informational molecules, he certainly was able to show by the biological properties of these agents that those biologic properties can change by how the agent is manipulated in different species. And probably variant CJD is an example of what has happened.

Interviewer: So do you think in a sense the misconceptions in the UK about, "Oh if it's scrapie gone into cattle it's going to act just like scrapie and it's not going to infect humans,." Do you think that could have been avoided if people had paid more attention to Richard Kimberlin's work or the work of others?

Richard: The whole world works on a pragmatic basis. You know, we deal with problems when they arise. We don't deal with problems because they might happen.

Pat: Why do you get vaccinated Rich?

Interviewer: Yeah, we always deal with problems because they might happen.

Richard: Oh no, you get vaccinated because you know that X number of people died of polio so –

Regina: Inductive reasoning.

Pat: What we were talking earlier.

Richard: Yeah. I mean, so people get vaccinated --

Pat: I can't decide on that because you haven't given me the evidence that it is required. You haven't proven to me that it is.

Richard: Yes you can.

Interviewer: But he was very insightful about the whole -- what I'm talking about now the -- oh yes, but the whole what it means to be science-based and how disease management is related.

Interviewer: Oh it's very intellectual. The philosophy of science.

Richard: I think he would give you a somewhat different perspective than we have.

Regina: How many people have died from this disease that we know nothing about?

Pat: I agree with you, I agree with you.

Regina: We don't know if this is brand new. We don't know if it's been around all along.

Pat: I won't give organs -- I won't sign an organ donor on myself.

Richard: As I've said when we did that ALS project, doing autopsies in those days was routine. Doing autopsies today is almost an impossibility. There are no autopsies done.

Pat: Who is going to look for PrP routinely?

Regina: The big one is the tonsils now. I mean, that is phenomenal to be able to do that. You have a whole pool to track them. It's like the Mormon genetic studies.

Regina: But how many people did they say it was Alzheimer's and it wasn't Alzheimer's?

Pat: It was CJD, right, because you don't look at the brain. You don't do any PrP testing, you don't do any -- and you have to do the brain.

Regina: So this could have been around a long time and we're just now realizing it.

Richard: Yes. There are some people who believe that variant CJD was around long before we recognized that it was there.

Interviewer: But you were saying earlier -- but sort of like even though it is something to worry about you're saying that it's still very low in frequency and death.

Regina: I mean auto accidents you have to worry about more.

Pat: But auto accidents you can do something about supposedly. You are in control and that's the main thing. You are in control here.

Regina: Not necessarily, if somebody falls asleep on the wheel, you're not in control of what they're doing. Again, there are so many things in life that you have to be concerned with.even though you don't want this to get out of hand. You don't want this to become epidemic. So you have to do something about it, yes, but how worried are you going to be about it again –

Richard: But Regina, the other side of that coin is that there are many things in life that you have absolutely no control over, but people feel that perhaps as far as variant CJD is concerned perhaps we do have control.

Regina: Yes I think they just developed a PrP knockout. I'm sure that is going to be a wonderful thing and you don't have to worry about transmission.

Pat: You know these are monster agents because you can't kill them. Oh my god it's going to eat me alive! Like the staph that can eat your skin, so there's a sense of no control. There's a sense of this boogey man coming at you.

Regina: That's right, it is scary.

Pat: I remember when I had my kids over in England when they brought the first DMs over there -- asked me to look at them and they were in there eating meat. We were there for two weeks and it was only later that I all of a sudden realized that they had been eating beef and I had my fingers crossed for a long time.

Regina: You worry about these things, but yet can you allow it to take over? No.

Pat: No, that was your chance. I mean you either got it or you didn't.

Interviewer: Presumably you were eating beef too, but you didn't worry about yourself. You were worried more about your kids.

Pat: No, because by this time what am I? I'm in my 40's. I'm in my 60's now.

Regina: Yeah that's young.

Pat: Listen -- yeah, but if I'm going to get CJD I'm going to be dead at 85. You go back to the old -- the real old infectivity studies on offspring hits in this depending on the age. But people get scared. They were doing a shooting here for one of the BBC channels and they wanted to see a droplet on a slide. So I put it on and I said, "Scrapie." "Ah, scrapie! Oh my God, I can't be in this room!" They were so afraid of being near these agents –

Regina: Pat, people in this building don't want to come down to the basement.

Pat: These agents are some of the most innocuous in one sense. They are the hardest to transmit. They are not like regular viruses. They don' t fly off in everything. You have to work hard to transmit these agents.

Regina: Right, even CJD.

Regina: So unless you inoculate yourself --

Pat: Right, you have to work hard. Dirty instruments.

Richard: I think nature takes pity on us every now and then, because if these agents were much more easily transmissible, there would be many more people infected.

Regina: Again, it's this incubation period and again you don't know --

Pat: May not be this type of disease. [laughs]

Interviewer: No, it's a possibility.

Pat: Very few people pay attention to Dickinson's work in the mice --

Regina: But I really think we have to stop. I don't care if it's sheep, cow --

Pat: I agree. I don't like getting –

Regina: -- giving all this to the salmon. Does the salmon have PrP. You know they they sterilized it and threw it into the salmon.

Pat: Yeah but it's not just salmon in the ocean.

Regina: So does salmon have PrP?

Pat: There are mammals in the ocean

Regina: But we don't know what replicates in salmon. How do we know? Did we ever test any?

Pat: I saw it happen with mercury and antibiotics and the rest of it.

Richard: It doesn't even have to replicate.

Regina: So do you think that mercury probably stops the replication?

Pat: I'm thinking of all the contamination, you know.

Interviewer: So mercury is good!

Regina: I'm going to be calling the butcher up to see if I can get chicken brain. We wanted to test chicken, and I had to track down a chicken brain.

Interviewer: Cool.

Richard: And chicken does have PrP. Interviewer: What were you saying about people forgetting Dickinson's sheep and mice work?

Pat: The point was the long incubation, short incubation, genetic differences and the breeding of a resistant strain of scrapie more or less -- to the common strain of scrapie, but more susceptible to another strain of scrapie, and the same with the mice. And the reason they forget about it is because it was pure genetics. It was not biochemical at all. So Stan will not use it and does not respect it. He was undercutting that a lot, earlier. He still is.

Interviewer: So for instance, things like the selective breeding programs in sheep and that's going on right on now both you in the US and the UK.

Pat: Yes.

Regina: I think it's very difficult to get a QQ sheet.

Interviewer: Right, everyone wants RR.

Richard: So maybe this will be some good that comes out of all of this that the best way to control these diseases may be through genetic susceptibility. You can't do that in the human population, but certainly you can do it in the animal population --

Pat: There's no resistant species except one that would not have the protein.

Regina: One that doesn't have the PrP period.

Pat: Yeah right. Then you have to ask the question, which would be a fascinating one to work with, take the null mice, inoculate them with an agent, pass it and pass it ten times and see what happens. That would be fascinating.

Pat: Graduate student project.

Interviewer: A long term graduate student.

Regina: -- you know Pat, you don't have to wait that long to do that, give it about a 200 incubation day.

Pat: Yeah give it 200 so you're not talking about contamination.

Richard: Yeah but where would you do the experiment?

Pat: You have to do it in a non-scrapie area.

Richard: Yeah, you can't do it here.

Pat: No I wouldn't do -- no, I would take the transgenic one.

Regina: Yeah but that would be something very interesting to do.

Interviewer: Another project. Keep in mind.

Regina: But there're so many.

Pat: You won't get money for that. You won't get money for anything like that.

Interviewer: Why not?

Regina: Well who's interested? I mean, we're trying to prove that maybe this is still alive and that's not the route and it's not the agent and nobody wants to hear that.

Pat: So why give money?

Regina: Right.

Regina: The pharmaceutical industry is not interested in that.

Pat: Because then it would undercut all the work with the null. Yes you don't ask questions anymore and try to answer them because the strains on the money are too great.

Interviewer: Are you're limited in what you can ask for?

Pat: Very much so.

Richard: That's not true just in this field, that's true in all biological science.

End of Interview